Dietary acrylamide intake and the risk of renal cell, bladder, and prostate cancer

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ABSTRACT

Background: Acrylamide, a probable human carcinogen, was recently detected in various heat-treated carbohydrate-rich foods. Epidemiologic studies on the relation with cancer have been few and largely negative.

Objective: We aimed to prospectively examine the association between dietary acrylamide intake and renal cell, bladder, and prostate cancers.

Design: The Netherlands Cohort Study on diet and cancer includes 120 852 men and women aged 55–69 y. At baseline (1986), a random subcohort of 5000 participants was selected for a case-cohort analysis approach using Cox proportional hazards analysis. Acrylamide intake was assessed with a food-frequency questionnaire at baseline and was based on chemical analysis of all relevant Dutch foods.

Results: After 13.3 y of follow-up, 339, 1210, and 2246 cases of renal cell, bladder, and prostate cancer, respectively, were available for analysis. Compared with the lowest quintile of acrylamide intake (mean intake: 9.5 μg/d), multivariable-adjusted hazard rates for renal cell, bladder, and prostate cancer in the highest quintile (mean intake: 40.8 μg/d) were 1.59 (95% CI: 1.09, 2.30; *P* for trend = 0.04), 0.91 (95% CI: 0.73, 1.15; *P* for trend = 0.60), and 1.06 (95% CI: 0.87, 1.30; *P* for trend = 0.69), respectively. There was an inverse nonsignificant trend for advanced prostate cancer in never smokers.

Conclusions: We found some indications for a positive association between dietary acrylamide and renal cell cancer risk. There were no positive associations with bladder and prostate cancer risk. *Am J Clin Nutr* 2008;87:1428–38.

INTRODUCTION

Acrylamide (C_3H_5NO) is a small hydrophilic molecule that polymerizes readily in the presence of an initiator because of the double bond between the first and second C-atoms, which makes it a versatile industrial chemical. In 1994, the International Agency for Research on Cancer classified acrylamide as a probable human carcinogen, on the basis of its carcinogenicity in rodents (1). Before 2002, acrylamide exposure was thought to occur mainly through occupational exposure, also through cigarette smoke, and to a minor extent through the consumption of water and the use of cosmetics. However, in 2002, Swedish scientists reported its presence in carbohydrate-rich foods that were produced at high temperatures, such as French fries and potato chips (2). This finding alarmed the scientific community, particularly because acrylamide is present in foods at considerably higher concentrations than are other well-known food carcinogens, such as polycyclic aromatic hydrocarbons and ethyl carbamate (3). Shortly after its discovery in food, acrylamide was shown to form in Maillard browning reactions, in which amino acids, particularly asparagine, react with reducing sugars at high temperatures (>120 °C) (4, 5).

The mechanism by which acrylamide causes cancer in laboratory animals and by which it may cause cancer in humans is as yet unclear (6); both genotoxic and nongenotoxic pathways have been suggested. Acrylamide itself and its epoxide metabolite glycidamide, which is generated by cytochrome P4502E1 (CYP2E1), are clastogenic, and glycidamide forms DNA adducts. As for possible nongenotoxic pathways, acrylamide reacts with glutathione and may thus influence the redox status of cells and gene transcription, or it may interfere with DNA repair or hormonal balances (6).

Animal studies have shown positive dose-response relations between acrylamide exposure and cancer in multiple organs in both mice and rats (7–10); included among those organs were several hormone-sensitive organs, such as the mammary glands and the uterus. Studies of occupational acrylamide exposure have been negative so far, apart from a finding of a greater risk of pancreatic cancer, but that finding was based on a small number of cases (11–16). Dietary acrylamide intake and its relation with various types of cancer have been studied in few case-control studies (17–19) and only 2 prospective cohort studies (20, 21). One prospective cohort study by our group showed a positive association between dietary acrylamide intake and risks of endometrial and ovarian cancers but not postmenopausal breast cancer (22).

Because the acrylamide molecule is small and hydrophilic, it passively diffuses throughout the body (23). For this reason, all tissues are theoretically targets for acrylamide carcinogenesis. In the present prospective cohort study, we investigated the relation between dietary acrylamide and risk of cancer of the kidney,

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bladder, and prostate. Prostate cancer was of particular interest because of its hormonal etiology.

SUBJECTS AND METHODS

Study cohort, cases, and follow-up

The Netherlands Cohort Study on diet and cancer (NLCS) began in September 1986 with the enrollment of 58 279 men and 62 573 women aged 55–69 y. At baseline, the participants completed a self-administered questionnaire on 150 food items and on possible risk factors for cancer, such as smoking, occupation, and physical activity.

Participants were informed that, by returning a completed questionnaire, they gave their consent to participate in a study of the etiology of cancer in relation to diet. The study protocol was approved by the Medical Ethics Committees of the University Hospital Maastricht and TNO Nutrition.

Data processing and analysis were based on the case-cohort approach, in which the cases were enumerated for the entire cohort (providing the numerator information for estimating incidence rates), whereas the accumulated person-years for the entire cohort were estimated from a subcohort of 5000 men and women randomly sampled from the entire cohort at baseline (providing the denominator information for estimating incidence rates). Thus, all of the incident cases in the total cohort were used in the analyses. The size of the subcohort was based on calculations that were described previously (24). Since the start of the study, vital status information was obtained from the subcohort at regular intervals. Incident cases in the total cohort have been detected by annual computerized record linkages to the regional cancer registries and the Netherlands Pathology Registry. The completeness of cancer follow-up through linkage with the cancer registries was estimated to be ≥96% (25). The follow-up of the subcohort was nearly 100% complete (only 2 male subcohort members were lost to follow-up) at the end of the follow-up period. Further details on the design of the study and methods of follow-up were presented elsewhere (24, 26–28).

The analyses are based on 13.3 y of follow-up, from September 1986 through December 1999. After the 13.3-y follow-up, there were 402, 1381, and 2599 incident, epithelial, microscopically confirmed cases of primary renal cell [International Classification of Diseases for Oncology (ICD-O)-3: C64], bladder (ICD-O-3: C67), and prostate (ICD-O-3: C61) carcinoma, respectively. There were 759 invasive bladder cancer cases and 734 papillary, noninvasive bladder cancer cases. Some bladder cancer cases had both an invasive and a papillary noninvasive tumor. As for prostate cancer, 947 cases, defined by tumor (T) and metastasis (M) status, were of the advanced prostate cancer type [which entails stage III prostate cancer (T3–4, M0) and stage IV prostate cancer (T0–4, M1); American Joint Committee on Cancer classification], and 1546 were of the localized type (T0–2, M0), whereas 106 cases had tumors of unknown stage.

Cases and subcohort members were excluded from analysis if they had been diagnosed with cancer (other than nonmelanoma skin cancer) at baseline and if their dietary data were incomplete or inconsistent. The selection and exclusion steps that resulted in the numbers of cases and subcohort members that were available for analysis are shown in **Figure 1**.

Acrylamide intake assessment

The NLCS food-frequency questionnaire (FFQ) contained questions on 150 food items (27). The acrylamide intake was estimated from the mean acrylamide concentration of food items and the frequency of consumption and portion size of the food items.

To obtain an intake estimate representative of the Dutch diet, we used data on acrylamide concentrations in food products on

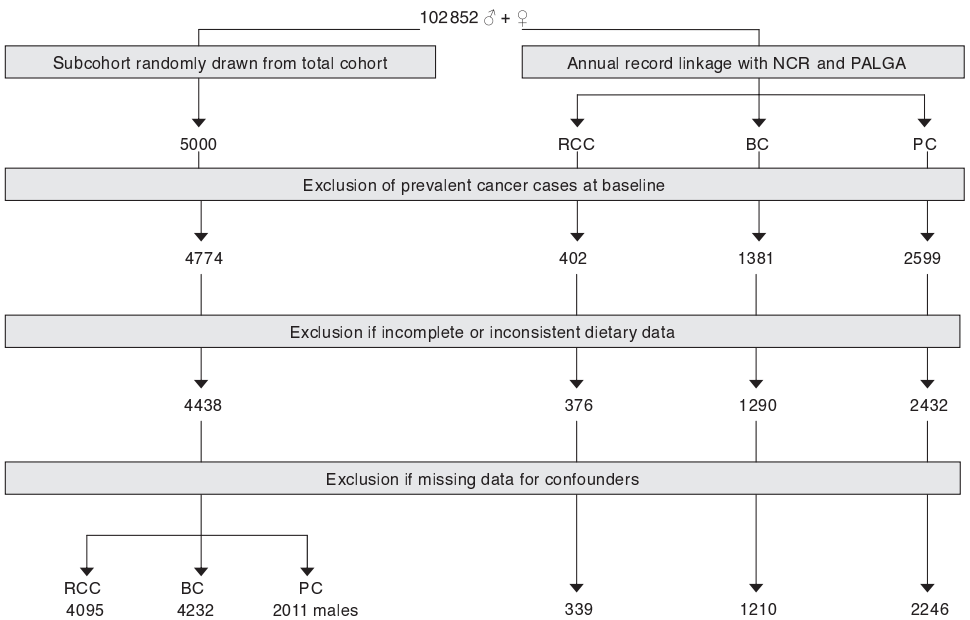

FIGURE 1. Flow diagram of subcohort members and cases on whom the analyses were based. NCR, the Netherlands Cancer Registry; PALGA, the Netherlands Pathology Registry; RCC, renal cell cancer; BC, bladder cancer; PC, prostate cancer.

the Dutch market. In 2002, the Dutch Food and Consumer Product Safety Authority used an elaborate sampling scheme to analyze acrylamide concentrations in various foods, such as bread, French fries, pastry and cake, Dutch spiced cake, potato chips, cornflakes, meat, fish, and several beverages (29). In 2005, more foods were analyzed to specifically accommodate the estimation of the acrylamide intake of the NLCS cohort; this series consisted of bread, rusk, specific types of cookies, rye bread, crisp bread, pastry and cake, chocolate and chocolate milk, nuts and salty snacks, peanut butter, and coffee (30). Bread was sampled and analyzed again in 2005, because the quantitation limit of the analytic method had decreased from 30 ppb in 2002 to 15 ppb in 2005, and that change offered the opportunity to more accurately estimate the acrylamide content of bread.

The foods that were used in the acrylamide intake assessment were assigned the mean value of the acrylamide values per food or a value one-half the quantitation limit when concentrations were lower than the quantitation limit (because even those foods probably do contain some acrylamide). Other foods (ie, meats, cooked and raw vegetables, and dairy products) were assigned the value zero. Because acrylamide concentrations in individual foods vary considerably, our group has performed a validation study to investigate whether using mean acrylamide concentrations in foods results in a sound estimate of total acrylamide intake (data not shown). The acrylamide content of 39 duplicate 24-h Dutch meals from 2004 was estimated by using the menu list (on which amounts of individual foods in the 24-h meals were listed) that the participants of the duplicate meal study filled in for their duplicate meals, and the same mean acrylamide concentrations for foods that were used in the NLCS study. Next, the acrylamide concentrations of the duplicate meals were chemically analyzed and correlated to the estimated acrylamide content, which rendered a Spearman correlation coefficient of 0.78. This finding indicates that it is feasible to make a sound rank ordering of the acrylamide intake via a 24-h meal by using these mean acrylamide concentrations for individual foods**.** The acrylamide concentrations of foods that were used in the acrylamide intake assessment are shown in **Table 1**.

TABLE 1
Acrylamide concentrations in foods used for the NLCS acrylamide intake assessment[1]

	Samples	Mean	Minimum	Maximum
	n	*μg/kg*	*μg/kg*	*μg/kg*
Potato chips	40	1249	310	2800
Dutch spiced cake	13	1018	260	1410
French fries	33	351	<LOQ[2]	1220
Salty snacks	12	277	45	867
Crisp bread	12	229	15	914
Cookies[3]	20	204	10	829
Cornflakes	12	121	<LOQ[2]	300
Peanut butter	2	113	107	118
Chocolate	6	60	22	116
Nuts	8	33	<LOQ[4]	83
Rusk	3	25	16	33
Rye bread	13	24	<LOQ[5]	60
Sweet pastry	19	18	<LOQ[4]	111
Coffee[6]	9	17	9	28
Chocolate milk	4	<LOQ[4]	<LOQ[4]	<LOQ[4]
Bread	22	<LOQ[4]	<LOQ[4]	<LOQ[4]

[1] NLCS, Netherlands Cohort Study; LOQ, limit of quantitation.
[2] LOQ in 2002 was 60 μg/kg.
[3] Measurements in cookies were performed in several types of cookies known to be eaten most frequently by the population comparable to that of the NLCS, according to information from the development phase of the questionnaire. Thus, an acrylamide concentration in cookies was based on the acrylamide concentration in the specific types of cookies weighted by the frequency of consumption by the NLCS-comparable population. The same was done for other composite food items, such as bread.
[4] LOQ in 2005 was 15 μg/kg.
[5] LOQ in 2002 was 30 μg/kg.
[6] Analyzed in brewed filtered coffee that was prepared according to general Dutch preparation instructions (7 g coffee powder/125 mL water).

Statistical analysis

Acrylamide was included in the statistical models as a continuous variable and as a quintile distribution. Furthermore, the fifth quintile was split into 2 deciles to investigate the highest 10% of the intake, which is more in line with the intake of current populations in developed countries (31), such as the Netherlands.

A priori, on the basis of the literature, variables, besides age and sex, were selected for inclusion in the multivariable-adjusted models. For renal cell cancer, those variables were hypertension, body mass index, energy intake, fruit consumption, and vegetable consumption; for bladder cancer, they were vegetable consumption, fruit consumption, tea consumption, and family history of bladder cancer; and for prostate cancer, they were socioeconomic status (SES), alcohol intake, and family history of prostate cancer (32). Other variables were included in the models if they changed the age- and sex-adjusted hazard ratios (HRs) of acrylamide (expressed as the interval between the 10th and 90th percentiles of intake: 27 μg acrylamide/d) by >10%. Different groups of variables were checked for confounding potential on the basis of this change of estimate rule. For renal cell cancer analysis, those variables were SES, physical activity, tea consumption, and a family history of renal cell cancer; for bladder cancer analysis, they were body mass index, SES, total fluid intake, energy intake, and meat, fish, and cheese intakes; and for prostate cancer analyses, they were body mass index, height, physical activity, energy intake, and meat, fish, vegetable, fruit, and dairy consumption. We also checked the following broad categories of foods or nutrients for confounding potential for the 3 cancer types: alcohol consumption, energy-adjusted intake of saturated fat, *trans* unsaturated fatty acids, carbohydrates, and dietary fiber. Because cigarette smoke is a very important source of acrylamide, smoking status (current or not current), the duration of smoking, and the number of cigarettes per day were included in the model for the total group (but not in the analyses for never smokers). Smokers have been shown to have, on average, 4 times the concentrations of acrylamide-hemoglobin adducts in their blood—a marker of the internal dose of acrylamide—as do nonsmokers (33, 34). For that same reason, subgroup analyses were performed for never smokers.

The proportional hazards assumption was tested by using scaled Schoenfeld residuals. HRs (and 95% CIs) were obtained by using Cox proportional hazards regression and STATA software (version 9.2; Stata Corp, College Station, TX). SEs were estimated by using the robust Huber-White sandwich estimator to account for additional variance introduced by sampling from the cohort. Tests for dose-response trends were performed by fitting the median acrylamide intake in each quintile for the

quintiles of acrylamide intake. To investigate whether the observed associations could be attributed to acrylamide itself or whether the association was due to other characteristics of acrylamide-containing foods than the acrylamide content, models were run with acrylamide and the foods (one at a time) that explained most variance in acrylamide intake.

Effect modification of the association between acrylamide intake and cancer by other variables was tested by using Wald chi-square tests. The variables that were tested for effect modification were selected on the basis of their ability to modify the activity of CYP2E1, the enzyme that converts acrylamide to glycidamide. Other acrylamide researchers have recommended studying the interaction between dietary acrylamide intake and factors that influence the expression of this enzyme in epidemiologic studies (35, 36). These variables are diabetes, obesity, smoking, alcohol consumption, and physical activity (35, 37–39). The categories of these variables were a priori based either on existing categories in the NLCS database (physical activity) or on the number of subcohort members within the categories, to strive for sufficient participants in each category (number of cigarettes smoked/d, number of smoking years, and alcohol intake). Throughout, 2-sided *P* values are reported.

RESULTS

On average, the subcohort members had a mean (±SD) daily acrylamide intake of 21.8 ± 12.0 μg/d, which corresponds to 0.30 ± 0.18 μg acrylamide · kg body $wt^{-1} \cdot d^{-1}$. Men had a slightly higher absolute intake (22.5 ± 12.1 μg/d) but a slightly lower intake per kg body wt (0.29 ± 0.16 μg · kg body $wt^{-1} \cdot d^{-1}$ than did women (absolute: 21.0 ± 11.9 μg/d; per kg body wt: 0.32 ± 0.19 μg · kg body $wt^{-1} \cdot d^{-1}$). Although coffee was overall the most important contributor to acrylamide intake, **Figure 2** shows that it was not coffee but, rather, Dutch spiced cake that was chiefly responsible for the variations in acrylamide intake in the population of the present study; next most responsible were coffee, French fries, potato chips, and cookies. These findings applied to both men and women.

The values of the covariables that were assessed for confounding potential for the subcohort and for the cases, separately for men and women, are shown in **Table 2**. There were no striking differences between cases and subcohort members for most of the variables. Only smoking (current smoking, number of cigarettes per day, and number of smoking years) was more prevalent among bladder cancer cases and among male renal cell cancer cases than among subcohort members.

The age- and multivariable-adjusted associations between acrylamide intake and renal cell cancer risk for men and women combined are shown in **Table 3**. There was a statistically significant positive association between acrylamide as a continuous variable and renal cell cancer risk in the group of men and women combined (HR: 1.10; 95% CI: 1.01, 1.21) per each 10-μg increment in acrylamide/d. For men and women separately, the HRs

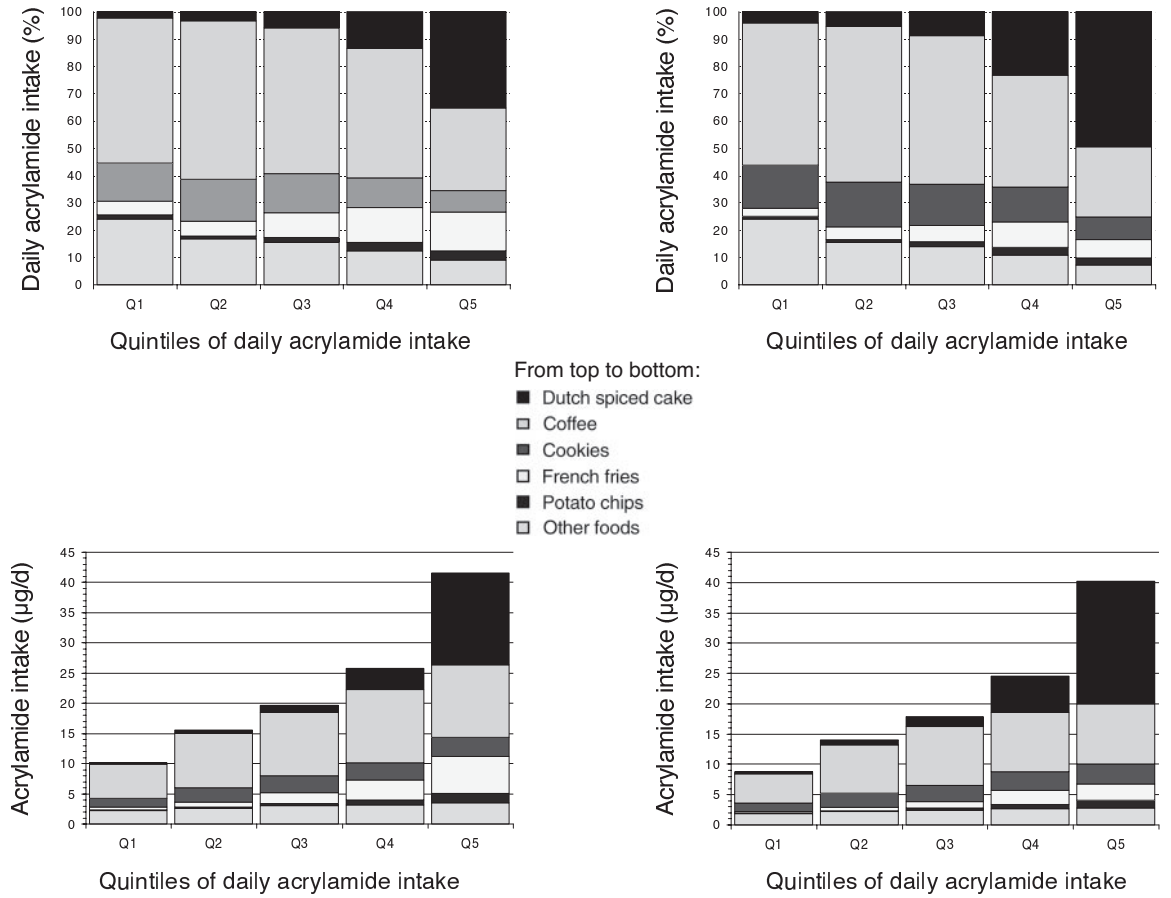

FIGURE 2. Relative and absolute contributions of foods to the mean daily dietary acrylamide intake of the present subcohort of the Netherlands Cohort Study. Left panels, men; right panels, women.

TABLE 2
Characteristics of cases and subcohort members in the Netherlands Cohort Study on diet and cancer (NLCS), 1986–1999

	Men				Women		
Variable	Subcohort[1] (n = 2191)[3]	Renal cell cancer cases (n = 241)	Bladder cancer cases (n = 1100)	Prostate cancer cases (n = 2432)	Subcohort[2] (n = 2247)	Renal cell cancer cases (n = 135)	Bladder cancer cases (n = 190)
Dietary variables							
Acrylamide intake (μg/d)	22.6 ± 12.2[4]	23.0 ± 11.8	23.0 ± 11.9	22.4 ± 11.7	21.0 ± 11.9	22.2 ± 13.8	19.9 ± 13.5
Acrylamide intake ($\mu g \cdot kg\ BW^{-1} \cdot d^{-1}$)	0.29 ± 0.16	0.30 ± 0.16	0.30 ± 0.16	0.29 ± 0.16	0.32 ± 0.19	0.32 ± 0.20	0.30 ± 0.21
Coffee (g/d)	578 ± 290	609 ± 264	620 ± 308	559 ± 260	497 ± 245	526 ± 253	484 ± 258
Dutch spiced cake (g/d)	4.1 ± 8.6	4.4 ± 8.9	4.2 ± 8.8	4.7 ± 9.0	5.7 ± 9.4	5.6 ± 10.5	5.1 ± 10.3
Cookies (g/d)	13.5 ± 10.6	13.1 ± 9.8	12.7 ± 12.0	14.1 ± 11.1	13.7 ± 11.0	15.1 ± 9.9	11.8 ± 13.6
Potato chips (g/d)	0.47 ± 1.72	0.52 ± 2.10	0.42 ± 1.67	0.43 ± 1.80	0.40 ± 1.93	0.45 ± 1.79	0.34 ± 1.33
French fries (g/d)	7.2 ± 15.4	6.2 ± 11.4	7.2 ± 13.3	5.9 ± 11.6	4.0 ± 8.7	5.0 ± 10.2	4.4 ± 10.4
Total energy intake (kcal)	2166 ± 511	2122 ± 479	2174 ± 484	2140 ± 490	1686 ± 398	1651 ± 388	1631 ± 381
Carbohydrate (g/d)	227 ± 66	223 ± 61	221 ± 60	222 ± 61	179 ± 48	177 ± 49	171 ± 50
Saturated fat (g/d)	36.9 ± 12.0	35.2 ± 10.7	37.3 ± 12.1	36.6 ± 12.0	29.8 ± 9.8	29.1 ± 9.5	29.3 ± 9.4
trans Unsaturated fatty acid (g/d)	3.3 ± 1.7	3.1 ± 1.6	3.3 ± 1.6	3.2 ± 1.6	2.5 ± 1.2	2.5 ± 1.2	2.4 ± 1.1
Fiber (g/d)	28.7 ± 8.7	29.1 ± 9.1	28.3 ± 7.9	28.7 ± 8.2	25.3 ± 7.0	25.2 ± 6.9	25.1 ± 7.4
Vegetables (g/d)	192 ± 85	198 ± 88	191 ± 80	192 ± 80	196 ± 81	186 ± 67	189 ± 80
Fruit (g/d)	154 ± 114	153 ± 114	150 ± 119	160 ± 110	196 ± 121	183 ± 109	194 ± 128
Dairy products (g/d)	309 ± 218	304 ± 214	299 ± 212	308 ± 200	299 ± 188	273 ± 178	285 ± 186
Meat (g/d)	106 ± 43	103 ± 45	110 ± 48	103 ± 42	93 ± 40	94 ± 39	93 ± 44
Fish (g/d)	14.2 ± 16.7	17.0 ± 19.6	14.7 ± 16.0	14.2 ± 16.4	11.7 ± 13.8	12.2 ± 15.1	11.3 ± 10.6
Alcohol (g/d)	15.0 ± 16.8	13.9 ± 15.1	17.5 ± 17.5	15.0 ± 16.4	5.9 ± 9.5	4.9 ± 8.7	6.7 ± 11.4
Tea (cups/d)[5]	2.5 ± 2.0	2.3 ± 1.9	2.4 ± 2.0	2.7 ± 2.0	3.0 ± 2.1	3.2 ± 2.3	3.0 ± 2.1
Nondietary variables							
Age (y)	61.3 ± 4.2	61.8 ± 3.8	62.3 ± 4.0	62.6 ± 4.0	61.4 ± 4.3	61.8 ± 4.0	62.1 ± 4.3
BMI (kg/m^2)	25.0 ± 2.6	25.3 ± 2.6	24.9 ± 2.5	25.0 ± 2.5	25.1 ± 3.6	25.8 ± 3.5	25.1 ± 3.6
Height (cm)	176 ± 7	177 ± 7	177 ± 7	176 ± 7	165 ± 6	166 ± 6	164 ± 6
Nonoccupational physical activity (min/d)	80 ± 68	78 ± 62	78 ± 65	78 ± 61	64 ± 53	62 ± 47	67 ± 62
Cigarettes (n/d)	14.7 ± 11.4	17.2 ± 12.9	16.9 ± 11.1	14.1 ± 11.6	4.6 ± 7.7	4.6 ± 8.0	7.2 ± 9.4
Smoking (y)	29.4 ± 15.8	31.9 ± 15.2	34.9 ± 14.0	28.9 ± 16.5	11.4 ± 15.8	11.0 ± 15.4	17.6 ± 18.4
Cigarette smoking							
Never smokers (%)	12.7	8.7	6.4	14.4	58.4	57.8	44.7
Former smokers (%)	51.6	50.2	46.5	53.2	20.6	20.0	23.2
Current smokers (%)	35.7	41.1	47.2	32.4	21.0	21.5	32.1
Education							
Primary school (%)	24.9	26.1	25.2	25.0	33.3	32.6	31.0
Lower vocational school (%)	20.6	22.8	19.9	17.6	23.1	25.2	17.0
Intermediate vocational or high school (%)	35.4	31.1	34.7	36.1	34.3	34.1	43.5
Higher vocational school or college (%)	18.6	19.5	19.4	20.6	8.8	7.4	8.5
Family history of renal cell cancer (%)	0.6	1.7	0.5	0.7	1.3	1.5	0.5
Family history of prostate cancer (%)	2.4	0.8	1.9	3.5	2.0	2.2	2.5
Family history of bladder cancer (%)	0.7	2.9	0.5	0.8	1.3	0.7	3.0

[1] Male subcohort contained 8 renal cell cancer cases, 40 bladder cancer cases, and 107 prostate cancer cases.
[2] Female subcohort contained 6 renal cell cancer cases and 7 bladder cancer cases.
[3] The number of subcohort members or cases after exclusion of participants with prevalent cancer at baseline, with incomplete or inconsistent dietary data, or both. The number of missing values varies for the variables in the table.
[4] $\bar{x}$ ± SD (all such values).
[5] 1 cup = 125 mL.

were similar, 1.10 (0.98, 1.23) and 1.11 (0.94, 1.31), respectively. In the group of men and women combined, there was a significant (P = 0.04) positive trend from the lowest to the highest quintile of acrylamide intake, with an HR of 1.59 (1.09, 2.30) in the highest quintile compared with the lowest. This pattern was observed in both men and women. There was no association in the subgroup of never smokers.

The associations between acrylamide intake and bladder cancer risk for men and women combined are presented in **Table 4**. Acrylamide intake was not associated with bladder cancer risk when male and female never, former, and current smokers were combined. Moreover, in the subgroup of participants within the highest decile of acrylamide intake, no greater risk was observed. However, in the never-smoking subgroup, acrylamide intake tended to be inversely associated with bladder cancer risk (P for trend = 0.07), but there was no linear dose-response relation. Stratification of the analyses by sex showed that an inverse association was found in women

TABLE 3
Association between dietary acrylamide intake and renal cell cancer risk; the Netherlands Cohort Study on diet and cancer (NLCS), 1986–1999[1]

	Overall				Never smokers			
	Cases	Person-years	HR (95% CI)[2]	HR (95% CI)[3]	Cases	Person-years	HR (95% CI)[2]	HR (95% CI)[4]
	n	*n*			*n*	*n*		
Acrylamide intake (10 μg/d)	339	49 600	1.07 (0.98, 1.16)	1.10 (1.01, 1.21)	93	19 202	1.08 (0.88, 1.31)	1.09 (0.89, 1.34)
Q1	56	9840	Ref (1.00)	Ref (1.00)	15	4189	Ref (1.00)	Ref (1.00)
Q2	66	9879	1.20 (0.83, 1.74)	1.25 (0.86, 1.83)	24	3930	1.73 (0.89, 3.36)	1.71 (0.87, 3.38)
Q3	76	9822	1.41 (0.98, 2.02)	1.48 (1.02, 2.15)	19	3384	1.67 (0.83, 3.34)	1.63 (0.80, 3.34)
Q4	62	10 077	1.14 (0.78, 1.66)	1.23 (0.83, 1.81)	15	3852	1.14 (0.54, 2.37)	1.15 (0.53, 2.49)
Q5	79	9981	1.43 (1.00, 2.04)	1.59 (1.09, 2.30)	20	3848	1.47 (0.74, 2.93)	1.51 (0.73, 3.10)
P for trend			0.12	0.04			0.73	0.68

[1] HR, hazard ratio; Q, quintile. The numbers of cases and person-years are the numbers that resulted after listwise deletion of observations with missing values for the selected confounders. HRs were calculated by using Cox proportional hazards analysis. The sex × acrylamide intake interaction was not significant.
[2] Adjusted for age and sex.
[3] Adjusted for age, sex, hypertension (yes or no), BMI, energy intake, fruit consumption, vegetable consumption, smoking status (current or not current), number of cigarettes per day, and number of years of smoking.
[4] Adjusted for age, sex, hypertension (yes or no), BMI, energy intake, fruit consumption, and vegetable consumption.

(although it was not statistically significant and did not have a linear dose-response relation), whereas, in men, the HRs were ≈1. The same pattern was found in never-smoking women. Similar results were observed for invasive bladder cancer.

From the data given in **Table 5**, it can be concluded that there were no indications of a positive association between acrylamide intake and total prostate cancer risk. There also was no greater HR in the highest decile of acrylamide intake. This finding was similar for advanced prostate cancer. However, there was an inverse statistically nonsignificant (*P* for trend = 0.10) dose-response relation between acrylamide intake and advanced prostate cancer risk in never smokers.

Adjustment for coffee intake slightly decreased the HRs of the highest quintiles of acrylamide intake and the continuous acrylamide variable for renal cell cancer. Adjustment for the other foods did not change the HRs (data not shown).

The results of the analyses (men and women combined) of interactions between acrylamide and possible CYP2E1-influencing variables are shown In **Table 6**, **Table 7**, and **Table**

TABLE 4
Association between dietary acrylamide intake and bladder cancer risk; the Netherlands Cohort Study on diet and cancer (NLCS), 1986–1999[1]

	Overall				Never smokers			
	Cases	Person-years	HR (95% CI)[2]	HR (95% CI)[3]	Cases	Person-years	HR (95% CI)[2]	HR (95% CI)[4]
	n	*n*			*n*	*n*		
Total bladder cancer								
Acrylamide intake (10 μg/d)	1210	51 111	1.01 (0.96, 1.07)	1.00 (0.95, 1.06)	155	19 903	0.90 (0.77, 1.06)	0.90 (0.76, 1.05)
Q1	249	10 108	Ref (1.00)	Ref (1.00)	52	4363	Ref (1.00)	Ref (1.00)
Q2	242	10 137	1.02 (0.82, 1.27)	0.96 (0.77, 1.20)	26	4060	0.58 (0.35, 0.98)	0.58 (0.34, 0.97)
Q3	229	10 121	1.00 (0.80, 1.24)	0.89 (0.71, 1.12)	24	3491	0.72 (0.42, 1.22)	0.73 (0.43, 1.25)
Q4	255	10 386	1.12 (0.90, 1.39)	1.01 (0.81, 1.26)	27	3989	0.69 (0.41, 1.15)	0.68 (0.40, 1.13)
Q5	235	10 360	0.97 (0.78, 1.21)	0.91 (0.73, 1.15)	26	4000	0.57 (0.34, 0.95)	0.55 (0.33, 0.93)
P for trend			0.93	0.60			0.08	0.07
Invasive bladder cancer								
Acrylamide intake (10 μg/d)	651	51 215	1.04 (0.97, 1.11)	1.03 (0.95, 1.11)	85	19 909	0.88 (0.70, 1.12)	0.88 (0.70, 1.12)
Q1	142	10 159	Ref (1.00)	Ref (1.00)	34	4363	Ref (1.00)	Ref (1.00)
Q2	127	10 158	0.95 (0.72, 1.25)	0.90 (0.68, 1.18)	16	4060	0.56 (0.30, 1.05)	0.56 (0.30, 1.06)
Q3	117	10 132	0.92 (0.69, 1.21)	0.81 (0.61, 1.08)	9	3491	0.43 (0.20, 0.91)	0.43 (0.20, 0.91)
Q4	122	10 404	0.96 (0.73, 1.26)	0.85 (0.64, 1.13)	10	3995	0.40 (0.19, 0.83)	0.40 (0.19, 0.83)
Q5	143	10 362	1.06 (0.81, 1.38)	1.00 (0.76, 1.31)	16	4000	0.54 (0.29, 1.01)	0.54 (0.28, 1.01)
P for trend			0.53	0.80			0.09	0.09

[1] HR, hazard ratio; Q, quintile. The numbers of cases and person-years are the numbers that resulted after listwise deletion of observations with missing values for the selected confounders. HRs were calculated through Cox proportional hazards analysis. The sex × acrylamide intake interaction was not significant.
[2] Adjusted for age and sex.
[3] Adjusted for age, sex, vegetable consumption, fruit consumption, tea consumption, bladder cancer in the family, smoking status (current or not current), number of cigarettes per day and number of years of smoking.
[4] Adjusted for age, sex, vegetable consumption, fruit consumption, tea consumption, and bladder cancer in the family.

TABLE 5
Association between dietary acrylamide intake and prostate cancer risk; the Netherlands Cohort Study on diet and cancer (NLCS), 1986–1999[1]

	Overall				Never smokers			
	Cases	Person-years	HR (95% CI)[2]	HR (95% CI)[3]	Cases	Person-years	HR (95% CI)[2]	HR (95% CI)[4]
	n	*n*			*n*	*n*		
Total prostate cancer								
Acrylamide intake (10 μg/d)	2246	23 208	1.01 (0.96, 1.06)	1.01 (0.96, 1.07)	347	3352	0.95 (0.83, 1.08)	0.95 (0.83, 1.09)
Q1	470	4631	Ref (1.00)	Ref (1.00)	101	784	Ref (1.00)	Ref (1.00)
Q2	473	4476	1.07 (0.88, 1.31)	1.07 (0.87, 1.31)	75	676	0.90 (0.56, 1.46)	0.84 (0.51, 1.38)
Q3	426	4761	1.00 (0.82, 1.23)	1.01 (0.82, 1.24)	53	553	0.88 (0.52, 1.50)	0.87 (0.50, 1.51)
Q4	406	4572	1.01 (0.82, 1.24)	1.02 (0.83, 1.26)	50	625	0.72 (0.42, 1.21)	0.73 (0.43, 1.24)
Q5	471	4769	1.05 (0.86, 1.29)	1.06 (0.87, 1.30)	68	715	0.75 (0.46, 1.22)	0.72 (0.43, 1.20)
P for trend			0.77	0.69			0.19	0.19
Advanced prostate cancer								
Acrylamide intake (10 μg/d)	741	23 208	1.02 (0.95, 1.09)	1.02 (0.95, 1.10)	117	3352	0.91 (0.75, 1.10)	0.90 (0.73, 1.11)
Q1	153	4631	Ref (1.00)	Ref (1.00)	34	784	Ref (1.00)	Ref (1.00)
Q2	166	4476	1.15 (0.88, 1.51)	1.15 (0.88, 1.51)	29	676	1.04 (0.55, 1.95)	0.98 (0.51, 1.88)
Q3	132	4761	0.95 (0.72, 1.26)	0.95 (0.71, 1.26)	17	553	0.86 (0.41, 1.78)	0.79 (0.38, 1.67)
Q4	133	4572	1.02 (0.77, 1.35)	1.02 (0.77, 1.36)	18	625	0.79 (0.39, 1.60)	0.76 (0.40, 1.57)
Q5	157	4769	1.08 (0.82, 1.41)	1.08 (0.82, 1.41)	19	715	0.63 (0.32, 1.23)	0.57 (0.27, 1.17)
P for trend			0.83	0.81			0.12	0.10

[1] HR, hazard ratio; Q, quintile. The numbers of cases and person-years are the numbers that resulted after listwise deletion of observations with missing values for the selected confounders. HRs were calculated through Cox proportional hazards analysis.
[2] Adjusted for age.
[3] Adjusted for age, socioeconomic status, prostate cancer in the family, alcohol intake, smoking status (current or not current), number of cigarettes per day, and number of years of smoking.
[4] Adjusted for age, socioeconomic status, prostate cancer in the family, and alcohol intake.

8. For renal cell cancer (Table 6), there were no significant *P* values for interaction for any of the possible CYP2E1-influencing variables. In the bladder cancer analyses (Table 7), cigarette smoking (ie, the number of cigarettes smoked/d) significantly modified the association between acrylamide and bladder cancer risk (*P* for interaction = 0.02), and there was a borderline statistically significant HR of 1.09 (1.00, 1.19) for persons smoking ≥15 cigarettes/d. Obese women had a lower acrylamide-associated risk of bladder cancer (HR: 0.47; 95% CI: 0.21, 1.05; *P* for interaction = 0.03) than did nonobese women, although this finding was based on only 16 obese cases (data not shown). The *P* values for interaction suggest that none of the variables statistically significantly modified the prostate cancer risk associated with acrylamide intake (Table 8).

DISCUSSION

This prospective cohort study provides indications that dietary acrylamide intake may increase the risk of renal cell cancer. In 2 case-control studies in Sweden, no association between acrylamide intake and renal cell cancer risk was observed (18, 19), but case-control studies may suffer from recall bias and selection bias, which may distort true associations. These types of biases are not relevant in the present prospective cohort study, which includes a nearly complete follow-up. In never or former smokers, there was no association between acrylamide and renal cell cancer risk, which may be explained by the higher risk in the subgroup with the longest duration of smoking (*see* Table 6). Thus, there may be a synergy between acrylamide intake and duration of smoking.

In the present study, acrylamide intake, overall, was not positively associated with a risk of bladder cancer, whether total or invasive. This finding is in line with the only study on this topic from Sweden. However, in that case-control study, the association was not evaluated separately for men and women. In the present study, there was a borderline statistically significant inverse trend but no clear linear dose-response relation for the association between acrylamide intake and bladder cancer risk in never smokers; this trend appeared to be restricted to the female subgroup. On the basis of the present study alone, we do not make a statement about whether there may be an inverse nonlinear dose-response relation between acrylamide intake and bladder cancer risk in women. It is too early to say whether the observed significantly decreased HRs in some quintiles of acrylamide intake represent a real biological effect or whether they are due to random fluctuations. More research on acrylamide intake and bladder cancer risk in women is needed. In contrast, there was a significant positive interaction between acrylamide intake and smoking in terms of the number of cigarettes smoked per day.

We found no positive association between acrylamide intake and prostate cancer risk. We did, however, observe an inverse linear trend in never smokers, but it was not statistically significant. In the only other epidemiologic study on dietary acrylamide intake and prostate cancer risk so far, no association was observed (17), but, in that case-control study, no subgroup analyses were performed for never smokers.

The relative risks of renal cell cancer associated with dietary acrylamide intake that we have observed in the present study are considerably higher than the relative risks that would be expected on the basis of linear extrapolation of the results of a cancer incidence study in rodents (40) that used the tissue that was most

TABLE 6
Acrylamide hazard ratios (HRs) (and 95% CIs) of renal cell cancer risk by strata of several covariables and tests for interaction: the Netherlands Cohort Study on diet and cancer (NLCS), 1986–1999[1]

Interaction variable	HRs per 10-μg/d increment of acrylamide	*P* for interaction
Smoking status		
Never ($n = 93$)	1.09 (0.89, 1.34)	0.83
Former ($n = 136$)	1.13 (0.99, 1.30)	
Current ($n = 110$)	1.08 (0.92, 1.27)	
Smoking (cigarettes/d)		0.83
0 ($n = 93$)	1.09 (0.89, 1.34)	
0 to <15 ($n = 100$)	1.11 (0.95, 1.31)	
≥15 ($n = 146$)	1.13 (0.99, 1.29)	
Smoking duration (y)		0.28
0 ($n = 93$)	1.09 (0.89, 1.34)	
0 to <30 ($n = 87$)	0.98 (0.82, 1.16)	
≥30 ($n = 159$)	1.21 (1.07, 1.37)	
Obesity (BMI; in kg/m^2)		0.22[2]
≤30		
All ($n = 322$)	1.11 (1.01, 1.22)	
Never smokers ($n = 84$)	1.08 (0.87, 1.34)	
>30		
All ($n = 17$)	0.90 (0.57, 1.43)	
Never smokers ($n = 9$)	—[3]	
BMI (kg/m^2)		
<20		
All ($n = 6$)	—	
Never smokers ($n = 1$)	—	
≥20–25		
All ($n = 161$)	1.13 (1.01, 1.27)	
Never smokers ($n = 36$)	0.98 (0.76, 1.27)	
>25		
All ($n = 172$)	1.06 (0.92, 1.23)	
Never smokers ($n = 56$)	1.18 (0.88, 1.60)	
Diabetes		0.43[2]
No		
All ($n = 322$)	1.11 (1.01, 1.22)	
Never smokers ($n = 88$)	1.11 (0.90, 1.37)	
Yes		
All ($n = 17$)	0.92 (0.61, 1.41)	
Never smokers ($n = 5$)	—	
Physical activity (min/d)		0.81[2] 0.54[4]
<30		
All ($n = 70$)	1.09 (0.91, 1.28)	
Never smokers ($n = 13$)	1.23 (0.80, 1.89)	
30–60		
All ($n = 98$)	1.09 (0.92, 1.30)	
Never smokers ($n = 36$)	0.93 (0.69, 1.25)	
60–90		
All ($n = 65$)	1.15 (0.92, 1.42)	
Never smokers ($n = 24$)	0.96 (0.68, 1.36)	
>90		
All ($n = 103$)	1.12 (0.93, 1.36)	
Never smokers ($n = 18$)	1.39 (0.84, 2.30)	
Alcohol intake (g/d)		0.52[2] 0.84[4]
0		
All ($n = 83$)	1.11 (0.93, 1.34)	
Never smokers ($n = 39$)	1.13 (0.81, 1.58)	
>0–5		
All ($n = 82$)	1.04 (0.85, 1.27)	
Never smokers ($n = 28$)	0.98 (0.69, 1.38)	
>5		
All ($n = 159$)	1.15 (1.01, 1.31)	
Never smokers ($n = 19$)	1.22 (0.85, 1.75)	

[1] HRs were calculated by using Cox proportional hazards analysis. Adjusted for age, sex, hypertension (yes or no), BMI, energy intake, fruit consumption, vegetable consumption, and (where appropriate) smoking status (current or not current), number of cigarettes/d, and number of years of smoking.
[2] *P* value for all subjects.
[3] An insufficient number of cases (all such).
[4] *P* value for never smokers only.

sensitive to carcinogenesis. In rodents, no tumors were observed in the kidney upon acrylamide administration (7–10). However, the results of extrapolating the high acrylamide dosages that were administered to animals to the low dosages that humans are exposed to through food are uncertain. The activity or capacity of enzymes in the metabolism of acrylamide may be either increased or decreased at low doses, and humans may differ from animals in their ability to otherwise detoxify or eliminate acrylamide or glycidamide.

The genotoxic action of glycidamide (35, 41, 42) is currently adopted as the mechanism of carcinogenic action in acrylamide cancer risk assessments. In a previous study, we observed positive associations between acrylamide intake and both endometrial and ovarian cancer risk (22), which might indicate that disturbance of hormonal balances may also be a mechanism of acrylamide carcinogenesis. Rodents may not be a good (quantitative) model for such hormonal effects.

The associations between acrylamide intake and renal cell, bladder, and prostate cancer risk that were observed in this study may be a reflection of the potential hormonal influences of acrylamide. A recent in vitro study of glycidamide-induced gene expression in human breast and colon cancer cells adds to this theory by showing up-regulation of genes that catalyze the conversion of inactive androgen and estrogen precursors to active forms, such as testosterone and 17β-estradiol (43). Renal cell cancer risk has been positively associated with estrogens in both animals and humans (44, 45). In prospective cohort studies, indications were found for an inverse association between estrogen and bladder cancer risk, in the form of inverse associations between age at menopause and bladder cancer risk and between lifetime years of ovulation and bladder cancer risk (46). In addition, both rodent and human bladder cells express estrogen receptors, and estrogens inhibit bladder carcinogenesis in rodents (46). If acrylamide were to increase the concentrations of free estrogen in the body, that could explain both its positive association with endometrial, ovarian cancer, and renal cell cancer risk and its negative association with bladder cancer risk in females. Furthermore, acrylamide has been shown to decrease concentrations of plasma testosterone in rats upon acrylamide injection into the rat brain (47). This finding may explain the decrease in HRs for prostate cancer in never smokers across the quintiles of acrylamide intake that was observed in the present study; the question of whether a high concentration of testosterone is indeed a risk factor for prostate cancer is, however, still under debate (48). In a prospective cohort study, in which the risk ratios for several hormones were mutually adjusted for each other and for sex hormone–binding globulin, testosterone was positively associated and estrogens were negatively associated with the risk of prostate cancer (49).

This study has some limitations. FFQs have limitations, as discussed elsewhere (50), but they are the only feasible way to assess dietary intake over a long period in large-scale epidemiologic studies. Acrylamide adducts to hemoglobin can be used as a biomarker of exposure (31), but they represent the exposure during the preceding 3 mo only. Furthermore, biomarkers are not specific with regard to the source of acrylamide. Thus, in biomarker studies of dietary acrylamide exposure, strict control for smoking and passive smoking is of the utmost importance. Moreover, the costs of using biomarkers limit the size of the population that can be used in a study. The NLCS FFQ has proved to be both valid (27) and reproducible (28) with respect to

TABLE 7
Acrylamide hazard ratios (HRs) (and 95% CIs) of bladder cancer risk by strata of several covariables and tests for interaction: the Netherlands Cohort Study on diet and cancer (NCLS), 1986–1999[1]

Interaction variable	HRs per 10-μg/d increment of acrylamide	*P* for interaction
Smoking status		0.19
Never (n = 155)	0.90 (0.77, 1.05)	
Former (n = 530)	1.06 (0.97, 1.15)	
Current (n = 525)	0.98 (0.89, 1.09)	
Smoking (cigarettes/d)		0.02
0 (n = 155)	0.90 (0.70, 1.05)	
0 to <15 (n = 384)	0.93 (0.84, 1.02)	
≥15 (n = 671)	1.09 (1.00, 1.19)	
Smoking duration (y)		0.23
0 (n = 155)	0.90 (0.77, 1.05)	
0 to <30 (n = 246)	0.99 (0.88, 1.10)	
≥30 (n = 809)	1.04 (0.96, 1.12)	
Obesity (BMI; in kg/m^2)		0.99[2]
		0.56[3]
≤30		
All (n = 1130)	1.01 (0.95, 1.07)	
Never smokers (n = 137)	0.93 (0.78, 1.10)	
>30		
All (n = 56)	1.00 (0.73, 1.37)	
Never smokers (n = 12)	0.79 (0.45, 1.40)	
BMI (kg/m^2)		0.40[2]
<20		
All (n = 22)	1.14 (0.69, 1.86)	
Never smokers (n = 3)	—[4]	
≥20–25		
All (n = 633)	0.98 (0.90, 1.06)	
Never smokers (n = 71)	0.85 (0.66, 1.09)	
>25		
All (n = 531)	1.04 (0.95, 1.14)	
Never smokers (n = 75)	1.00 (0.80, 1.25)	
Diabetes		0.18[2]
No		
All (n = 1163)	1.01 (0.95, 1.08)	
Never smokers (n = 148)	0.90 (0.77, 1.06)	
Yes		
All (n = 47)	0.72 (0.46, 1.10)	
Never smokers (n = 7)	—	
Physical activity (min/d)		0.87[2]
		0.11[3]
<30		
All (n = 244)	0.95 (0.84, 1.08)	
Never smokers (n = 45)	0.86 (0.68, 1.07)	
30–60		
All (n = 356)	1.01 (0.90, 1.14)	
Never smokers (n = 43)	0.64 (0.43, 0.96)	
60–90		
All (n = 229)	1.00 (0.86, 1.16)	
Never smokers (n = 28)	1.28 (0.87, 1.91)	
>90		
All (n = 376)	1.03 (0.92, 1.14)	
Never smokers (n = 37)	0.99 (0.70, 1.41)	
Alcohol intake (g/d)		0.67[2]
		0.56[3]
0		
All (n = 146)	1.06 (0.92, 1.23)	
Never smokers (n = 39)	0.73 (0.51, 1.02)	
>0–5		
All (n = 265)	0.98 (0.87, 1.11)	
Never smokers (n = 62)	0.97 (0.73, 1.30)	
>5		
All (n = 783)	0.99 (0.91, 1.07)	
Never smokers (n = 48)	0.93 (0.70, 1.23)	

[1] HRs were calculated by using Cox proportional hazards analysis. Adjusted for age, sex, vegetable consumption, fruit consumption, tea consumption, family history of bladder cancer, and (where appropriate) smoking status (current or not current), number of cigarettes/d, and number of years of smoking.
[2] *P* values for all subjects.
[3] *P* values for never smokers only.
[4] An insufficient number of cases (all such).

TABLE 8
Acrylamide hazard ratios (HRs) (and 95% CIs) of prostate cancer risk by strata of several covariables and tests for interaction: the Netherlands Cohort Study on diet and cancer (NCLS), 1986–1999[1]

Interaction variable	HRs per 10-μg/d increment of acrylamide	*P* for interaction
Smoking status		0.26
Never (n = 347)	0.95 (0.82, 1.09)	
Former (n = 1204)	1.06 (0.99, 1.14)	
Current (n = 695)	0.96 (0.87, 1.06)	
Smoking (cigarettes/d)		0.39
0 (n = 347)	0.95 (0.82, 1.09)	
0 to <15 (n = 804)	1.05 (0.97, 1.13)	
≥15 (n = 1095)	1.00 (0.92, 1.09)	
Smoking duration (y)		0.48
0 (n = 347)	0.95 (0.82, 1.09)	
0 to <30 (n = 642)	1.06 (0.96, 1.16)	
≥30 (n = 1257)	1.01 (0.94, 1.08)	
Obesity		0.52[2]
No		
All (n = 2122)	1.02 (0.96, 1.07)	
Never smokers (n = 327)	0.97 (0.84, 1.13)	
Yes		
All (n = 67)	1.13 (0.78, 1.63)	
Never smokers (n = 9)	—[3]	
BMI (kg/m^2)		0.44[2]
<20		
All (n = 34)	2.64 (1.28, 5.47)	
Never smokers (n = 9)	—	
≥20–25		
All (n = 1123)	1.03 (0.96, 1.11)	
Never smokers (n = 179)	1.06 (0.85, 1.31)	
<25		
All (n = 1032)	1.00 (0.93, 1.08)	
Never smokers (n = 148)	0.85 (0.68, 1.08)	
Diabetes		0.99[2]
		0.12[4]
No		
All (n = 2175)	1.02 (0.96, 1.07)	
Never smokers (n = 335)	0.94 (0.82, 1.08)	
Yes		
All (n = 71)	0.88 (0.58, 1.34)	
Never smokers (n = 12)	1.97 (0.72, 5.35)	
Physical activity (min/d)		0.20[2]
		0.09[4]
<30		
All (n = 351)	0.92 (0.82, 1.03)	
Never smokers (n = 54)	0.73 (0.51, 1.05)	
30–60		
All (n = 673)	1.03 (0.93, 1.14)	
Never smokers (n = 91)	0.77 (0.59, 1.00)	
60–90		
All (n = 502)	0.96 (0.84, 1.10)	
Never smokers (n = 85)	0.97 (0.68, 1.40)	
>90		
All (n = 698)	1.09 (0.99, 1.20)	
Never smokers (n = 114)	1.26 (0.94, 1.69)	
Alcohol intake (g/d)		0.21[2]
		0.49[4]
0		
All (n = 310)	0.99 (0.88, 1.12)	
Never smokers (n = 97)	0.94 (0.73, 1.20)	
>0–5		
All (n = 488)	1.12 (1.01, 1.24)	
Never smokers (n = 104)	1.17 (0.89, 1.54)	
>5		
All (n = 1448)	0.99 (0.92, 1.16)	
Never smokers (n = 146)	0.88 (0.70, 1.10)	

[1] HRs were calculated by using Cox proportional hazards analysis. Adjusted for age, socioeconomic status, family history of prostate cancer, alcohol intake, and (where appropriate) smoking status (current or not current), number of cigarettes/d, and number of years of smoking.
[2] *P* value for all subjects.
[3] An insufficient number of cases (all such).
[4] *P* value for never smokers only.

nutrients that correlate with acrylamide, such as carbohydrates and fiber. The acrylamide intake assessment is an important asset of the present study, as are its large study size and prospective nature, the latter of which excludes selection and recall bias. We used acrylamide concentrations in foods from the Dutch market only, and we specifically sampled and analyzed foods that were relevant for the NLCS population.

Within the validation study that we described in the Methods section, visual inspection of the scatter plot and the Bland-Altman plot showed that most of the estimated amounts were higher than the corresponding measured amounts. This finding implies that the use of mean values of acrylamide concentrations in foods to estimate dietary acrylamide intake on a continuous scale probably leads to some underestimation of the true acrylamide-associated risk.

It has to be borne in mind that the variation in acrylamide intake in our study was to a large extent due to Dutch spiced cake, which resembles gingerbread of the cake-like, noncrusty type. Dutch spiced cake does not necessarily contain ginger, but it contains other spices and sometimes fruit. Concentrations of acrylamide in Dutch spiced cake (mean: 1018 ppb) are much higher than those in plain cake (mean: <30 ppb). Because of the addition of glucose and fructose syrup, Dutch spiced cake contains a greater amount of reducing sugars than do other cakes. In the presence of ammonium hydrogen carbonate, which is often used as a baking agent in spiced cake, the formation of acrylamide is further enhanced (51). There is no indication that the associations between acrylamide intake and renal cell, bladder, and prostate cancer risk should be attributed to other ingredients of spiced cake; the results did not change signficantly after adjustment for spiced cake.

For the interaction analyses, we calculated many HRs in many small subgroups, which makes it likely that some of the observed significant *P* values for interaction or HRs in subgroups were spurious. Therefore, they should be interpreted cautiously, and their analysis should be repeated in other epidemiologic studies.

Current populations, especially young people, consume larger amounts of potato chips and French fries than did the NLCS population. Because of the high acrylamide concentrations of these foods, current dietary intake of acrylamide is assumed to be considerably higher (29, 31, 52) than the intake in the present study. In contrast, industry and science are investigating ways to reduce acrylamide concentrations in food and have succeeded to some extent with respect to certain foods (53).

Although replication in other prospective studies is needed, the present study suggests that acrylamide is not neutral with regard to carcinogenesis. Depending on the tissue, acrylamide may have both carcinogenic and anticarcinogenic properties, which suggests that the genotoxic action of glycidamide, the epoxide metabolite of acrylamide, may not be the (only) pathway through which acrylamide exerts its effects. The results of the interaction analyses between acrylamide and the CYP2E1-modifying variables also do not clearly point toward a glycidamide-mediated genotoxic effect, because they do not modify, or at least do not consistently modify, the association between acrylamide intake and renal cell, bladder, and prostate cancer risk.

We encourage other researchers to prospectively investigate the association between dietary acrylamide intake and renal, bladder, and prostate cancer risk; to perform subgroup analyses for nonsmokers; and to study interactions with factors that modify CYP2E1 activity. In the mean time, the indications of a positive association between acrylamide intake and endometrial, ovarian, and renal cell cancer risk should be an incentive for food industries to continue striving to lower acrylamide concentrations in foods.

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The authors' responsibilities were as follows—JGH: designed the study, collected the data, analyzed the data, interpreted the results, and drafted the manuscript; LJS, EJK, RAG, and PAvdB: designed the study, collected the data, contributed to the drafting of the manuscript, and approved the final version; LJS, RAG, and PAvdB: obtained funding; and PAvdB: study supervision. EJK is an employee of the governmental Dutch Food and Consumer Product Safety Authority but reported no conflict of interest. None of the other authors had a personal or financial conflict of interest.

REFERENCES

1. Monographs on the evaluation of carcinogen risk to humans: some industrial chemicals. Lyon, France: International Agency for Research on Cancer, 1994.
2. Tareke E, Rydberg P, Karlsson P, Eriksson S, Tornqvist M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. J Agric Food Chem 2002;50:4998–5006.
3. Summary and conclusions of the sixty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Geneva, Switzerland: JECFA, 2005.
4. Mottram DS, Wedzicha BL, Dodson AT. Acrylamide is formed in the Maillard reaction. Nature 2002;419:448–9.
5. Stadler RH, Blank I, Varga N, et al. Acrylamide from Maillard reaction products. Nature 2002;419:449–50.
6. Besaratinia A, Pfeifer GP. A review of mechanisms of acrylamide carcinogenicity. Carcinogenesis 2007;28:519–28.
7. Johnson KA, Gorzinski SJ, Bodner KM, et al. Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fischer 344 rats. Toxicol Appl Pharmacol 1986;85:154–68.
8. Bull RJ, Robinson M, Laurie RD, et al. Carcinogenic effects of acrylamide in Sencar and A/J mice. Cancer Res 1984;44:107–11.
9. Bull RJ, Robinson M, Stober JA. Carcinogenic activity of acrylamide in the skin and lung of Swiss-ICR mice. Cancer Lett 1984;24:209–12.
10. Friedman MA, Dulak LH, Stedham MA. A lifetime oncogenicity study in rats with acrylamide. Fundam Appl Toxicol 1995;27:95–105.
11. Marsh GM, Lucas LJ, Youk AO, Schall LC. Mortality patterns among workers exposed to acrylamide: 1994 follow up. Occup Environ Med 1999;56:181–90.
12. Schulz MR, Hertz-Picciotto I, van Wijngaarden E, Hernandez JC, Ball LM. Dose-response relation between acrylamide and pancreatic cancer. Occup Environ Med 2001;58:609.
13. Sobel W, Bond GG, Parsons TW, Brenner FE. Acrylamide cohort mortality study. Br J Ind Med 1986;43:785–8.
14. Collins JJ, Swaen GM, Marsh GM, Utidjian HM, Caporossi JC, Lucas LJ. Mortality patterns among workers exposed to acrylamide. J Occup Med 1989;31:614–7.
15. Swaen GM, Haidar S, Burns CJ, et al. Mortality study update of acrylamide workers. Occup Environ Med 2007;64:396–401 (Epub 2007 Jan 25).
16. Marsh GM, Youk AO, Buchanich JM, Kant IJ, Swaen G. Mortality patterns among workers exposed to acrylamide: updated follow up. J Occup Environ Med 2007;49:82–95.

17. Pelucchi C, Galeone C, Levi F, et al. Dietary acrylamide and human cancer. Int J Cancer 2006;118:467–71.
18. Mucci LA, Dickman PW, Steineck G, Adami HO, Augustsson K. Dietary acrylamide and cancer of the large bowel, kidney, and bladder: absence of an association in a population-based study in Sweden. Br J Cancer 2003;88:84–9.
19. Mucci LA, Lindblad P, Steineck G, Adami HO. Dietary acrylamide and risk of renal cell cancer. Int J Cancer 2004;109:774–6.
20. Mucci LA, Adami HO, Wolk A. Prospective study of dietary acrylamide and risk of colorectal cancer among women. Int J Cancer 2006;118:169–73.
21. Mucci LA, Sandin S, Balter K, Adami HO, Magnusson C, Weiderpass E. Acrylamide intake and breast cancer risk in Swedish women. JAMA 2005;293:1326–7.
22. Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA. A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian and breast cancer. Cancer Epidemiol Biomarkers Prev 2007;16:2304–13.
23. Friedman M. Chemistry, biochemistry, and safety of acrylamide. A review. J Agric Food Chem 2003;51:4504–26.
24. van den Brandt PA, Goldbohm RA, van 't Veer P, Volovics A, Hermus RJ, Sturmans F. A large-scale prospective cohort study on diet and cancer in the Netherlands. J Clin Epidemiol 1990;43:285–95.
25. Goldbohm RA, van den Brandt PA, Dorant E. Estimation of the coverage of Dutch municipalities by cancer registries and PALGA based on hospital discharge data. Tijdschr Soc Gezondheidsz 1994;72:80–84.
26. van den Brandt PA, Schouten LJ, Goldbohm RA, Dorant E, Hunen PM. Development of a record linkage protocol for use in the Dutch Cancer Registry for Epidemiological Research. Int J Epidemiol 1990;19:553–8.
27. Goldbohm RA, van den Brandt PA, Brants HA, et al. Validation of a dietary questionnaire used in a large-scale prospective cohort study on diet and cancer. Eur J Clin Nutr 1994;48:253–65.
28. Goldbohm RA, van 't Veer P, van den Brandt PA, et al. Reproducibility of a food frequency questionnaire and stability of dietary habits determined from five annually repeated measurements. Eur J Clin Nutr 1995;49:420–9.
29. Konings EJ, Baars AJ, van Klaveren JD, et al. Acrylamide exposure from foods of the Dutch population and an assessment of the consequent risks. Food Chem Toxicol 2003;41:1569–79.
30. Database of the European Commission Institute for Reference Materials and Measurements (IRMM). Internet: http://www.irmm.jrc.be/html/activities/acrylamide/database.htm (accessed 3 August 2006).
31. Dybing E, Farmer PB, Andersen M, et al. Human exposure and internal dose assessments of acrylamide in food. Food Chem Toxicol 2005;43:365–410.
32. Schottenfeld D, Fraumeni JF Jr. Cancer epidemiology and prevention. 3rd ed. New York, NY: Oxford University Press, 2006.
33. Schettgen T, Rossbach B, Kutting B, Letzel S, Drexler H, Angerer J. Determination of haemoglobin adducts of acrylamide and glycidamide in smoking and non-smoking persons of the general population. Int J Hyg Environ Health 2004;207:531–9.
34. Bergmark E. Hemoglobin adducts of acrylamide and acrylonitrile in laboratory workers, smokers and nonsmokers. Chem Res Toxicol 1997;10:78–84.
35. Ghanayem BI, Witt KL, Kissling GE, Tice RR, Recio L. Absence of acrylamide-induced genotoxicity in CYP2E1-null mice: evidence consistent with a glycidamide-mediated effect. Mutat Res 2005;578:284–97.
36. Lamy E, Volkel Y, Roos PH, Kassie F, Mersch-Sundermann V. Ethanol enhanced the genotoxicity of acrylamide in human, metabolically competent HepG2 cells by CYP2E1 induction and glutathione depletion. Int J Hyg Environ Health 2008;211:74–81 (Epub 2007 Jul 30).
37. Ardies CM, Smith TJ, Kim S, Yang CS. Induction of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) activation in rat lung microsomes by chronic ethanol consumption and repeated running exercise. Cancer Lett 1996;103:209–18.
38. Howard LA, Micu AL, Sellers EM, Tyndale RF. Low doses of nicotine and ethanol induce CYP2E1 and chlorzoxazone metabolism in rat liver. J Pharmacol Exp Ther 2001;299:542–50.
39. Wang Z, Hall SD, Maya JF, Li L, Asghar A, Gorski JC. Diabetes mellitus increases the in vivo activity of cytochrome P450 2E1 in humans. Br J Clin Pharmacol 2003;55:77–85.
40. Dybing E, Sanner T. Risk assessment of acrylamide in foods. Toxicol Sci 2003;75:7–15.
41. Gamboa da Costa G, Churchwell MI, Hamilton LP, et al. DNA adduct formation from acrylamide via conversion to glycidamide in adult and neonatal mice. Chem Res Toxicol 2003;16:1328–37.
42. Doerge DR, da Costa GG, McDaniel LP, Churchwell MI, Twaddle NC, Beland FA. DNA adducts derived from administration of acrylamide and glycidamide to mice and rats. Mutat Res 2005;580:131–41.
43. Clement FC, Dip R, Naegeli H. Expression profile of human cells in culture exposed to glycidamide, a reactive metabolite of the heat-induced food carcinogen acrylamide. Toxicology 2007;240:111–24.
44. Tanaka Y, Sasaki M, Kaneuchi M, Fujimoto S, Dahiya R. Estrogen receptor alpha polymorphisms and renal cell carcinoma–a possible risk. Mol Cell Endocrinol 2003;202:109–16.
45. Nicodemus KK, Sweeney C, Folsom AR. Evaluation of dietary, medical and lifestyle risk factors for incident kidney cancer in postmenopausal women. Int J Cancer 2004;108:115–21.
46. Prizment AE, Anderson KE, Harlow BL, Folsom AR. Reproductive risk factors for incident bladder cancer: Iowa Women's Health Study. Int J Cancer 2006;120:1093–8.
47. Ali SF, Hong JS, Wilson WE, Uphouse LL, Bondy SC. Effect of acrylamide on neurotransmitter metabolism and neuropeptide levels in several brain regions and upon circulating hormones. Arch Toxicol 1983;52:35–43.
48. Platz EA, Giovannucci E. The epidemiology of sex steroid hormones and their signaling and metabolic pathways in the etiology of prostate cancer. J Steroid Biochem Mol Biol 2004;92:237–53.
49. Gann PH, Hennekens CH, Ma J, Longcope C, Stampfer MJ. Prospective study of sex hormone levels and risk of prostate cancer. J Natl Cancer Inst 1996;88:1118–26.
50. Konings EJ, Hogervorst JGF, Schouten LJ, van den Brandt PA. Assessing exposure levels of acrylamide. In: Skog K, Alexander J, eds. Acrylamide and other hazardous compounds in heat-treated foods. Cambridge, United Kingdom: Woodhead Publishing Limited, 2006:214–25.
51. Amrein TM, Andres L, Manzardo GG, Amado R. Investigations on the promoting effect of ammonium hydrogencarbonate on the formation of acrylamide in model systems. J Agric Food Chem 2006;54:10253–61.
52. Matthys C, Bilau M, Govaert Y, Moons E, De Henauw S, Willems JL. Risk assessment of dietary acrylamide intake in Flemish adolescents. Food Chem Toxicol 2005;43:271–8.
53. Confederation of the Food and Drink Industries in the Euopean Union (CIAA). Acrylamide status report December 2004: a summary of the efforts and progress achieved to date by the European food and drink industry (CIAA) in lowering levels of acrylamide in food. Brussels, Belgium: CIAA, 2004.